Genetic Profiles Related to Pathogenesis in Sporadic Intracranial Aneurysm Patients

Quan Cheng[1,9], Zhenyan Li[1], Ruizhe Wang[3], Hongfu Zhang[4], Hui Cao[5], Fenghua Chen[1], Huangbao Li[6], Zhiwei Xia[7], Songshan Feng[1], Hao Zhang[1], Yuhua Rui[8], Fan Fan[1,2]

BACKGROUND: **Intracranial aneurysm (IA) represents a cerebrovascular disorder that featured by dilation or bulging of the weakened blood vessel wall. When it ruptures, an IA leads to subarachnoid hemorrhage with high disability and mortality rates. Despite the numerous studies focusing on IA ruptures, little research on IA pathogenesis has been reported. In this study, we aimed to reveal key genes related to IA formation.**

METHODS: **Four datasets from Gene Expression Omnibus data were downloaded, normalized, and separated into the IA group and the normal vessel control group for analyses. We screened for differentially expressed genes (DEGs) between groups and conducted functional enrichment, pathway enrichment, and gene set enrichment analysis analyses among significant DEGs.**

RESULTS: **according to our analyses, significant DEGs majorly associate with smooth muscle system and the complement system. Among all DEGs, 5 down-regulated genes (*MYH11*, *CNN1*, *MYOCD*, *ACTA1*, and *LMOD1*) and 3 up-regulated genes (*C1QB*, *C3AR1*, and *VSIG4*) are most relevant in IA formation.**

CONCLUSIONS: **Key DEGs identified in this study are related to IA pathogenesis. Among identified DEGs, *LMOD1* is the most significant and merits more attention.**

INTRODUCTION

Intracranial aneurysms (IAs), characterized by weaknesses in the endothelial layer and dilatations of intracranial arteries, is a leading cause of subarachnoid hemorrhage and thus is associated with morbidity and mortality rates.[1] Therefore, surgical or interventional coiling is recommended to treat IAs.[2] The worldwide incidence rate of IAs is approximately 3.2%; most IAs will not rupture or cause subarachnoid hemorrhage, however.[3]

Smooth muscle cells (SMCs) play pivotal roles in the formation, progression, and rupture of IAs.[4] SMCs in the cerebral artery wall have 2 major functions: mediating the synthesis and turnover of extracellular matrix proteins necessary to sustain the integrity of vessels in structure, and regulating luminal diameter to increase or decrease blood flow. The loss of SMCs is characteristic in arterial remodeling before IA formation and rupture.[5]

Although microarray sequencing technologies have powerfully expanded our understanding of tumor biology, much less research has focused on the genetic pathogenesis of IAs compared with intracranial tumors. In this study, converging data from online databases, we focused mainly on genetic profiles of patients with IAs to look for potential genetic genes involved in the pathogenesis of IA.

MATERIALS AND METHODS

Analysis Overview

In this study, 4 IA datasets were downloaded and reanalyzed: GSE75436, GSE6551, GSE26969, and GSE13353 (platform GPL570) from the National Center of Biotechnology Information Gene

Key words
- Bioinformatics
- Intracranial aneurysm
- Pathogenesis

Abbreviations and Acronyms
DEG: Differentially expressed gene
GO: Gene Ontology
GSEA: Gene Set Enrichment Analysis
IA: Intracranial aneurysm
KEGG: Kyoto Encyclopedia of Genes and Genomes
PPI: Protein–protein interaction
SMC: Smooth muscle cell

From the [1]Department of Neurosurgery, Xiangya Hospital, Central South University, Changsha; [2]Center for Medical Genetics & Hunan Provincial Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha; [3]Department of Urinary Surgery, Xiangya Hospital, Central South University, Changsha; [4]Department of Neurosurgery, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou; [5]Department of Psychiatry, The Second People's Hospital of Hunan University of Chinese Medicine, Changsha; [6]Department of Hepatobiliary and Pancreatic Surgery, First Hospital of Jiaxing University, Zhejiang; [7]Department of Neurology, Xiangya Hospital, Central South University, Changsha; [8]Department of Ophthalmology, Xiangya Hospital, Central South University, Changsha; and [9]Department of Clinical Pharmacology, Xiangya Hospital, Central South University, Changsha, China

To whom correspondence should be addressed: Fan Fan, M.D. [E-mail: fanfan@csu.edu.cn]

Quan Cheng and Zhenyan Li are co-first-authors.

Citation: World Neurosurg. (2019) 131:e23-e31. https://doi.org/10.1016/j.wneu.2019.06.110

Journal homepage: www.journals.elsevier.com/world-neurosurgery

Available online: www.sciencedirect.com

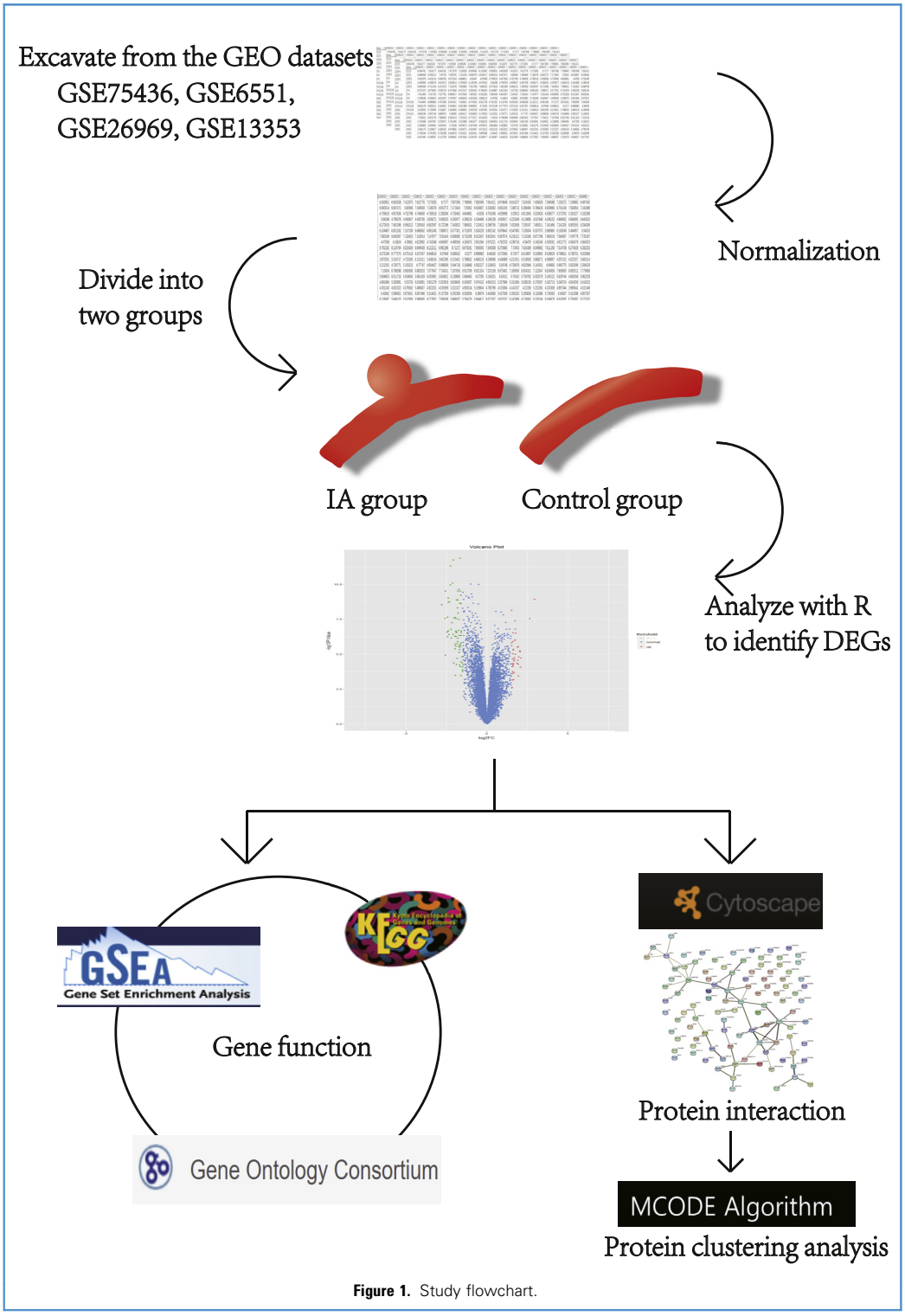

Figure 1. Study flowchart.

Expression Omnibus, the most widely acknowledged gene expression resource for scientific community submitted data. All samples in these datasets were from either cadavers or human patients obtained with proper consent. Once the 4 datasets were normalized to eliminate batch effects, they were divided into 2 groups, the IA group and the control group. Differentially expressed genes (DEGs) between IAs and control arteries were screened using R software (R Foundation for Statistical

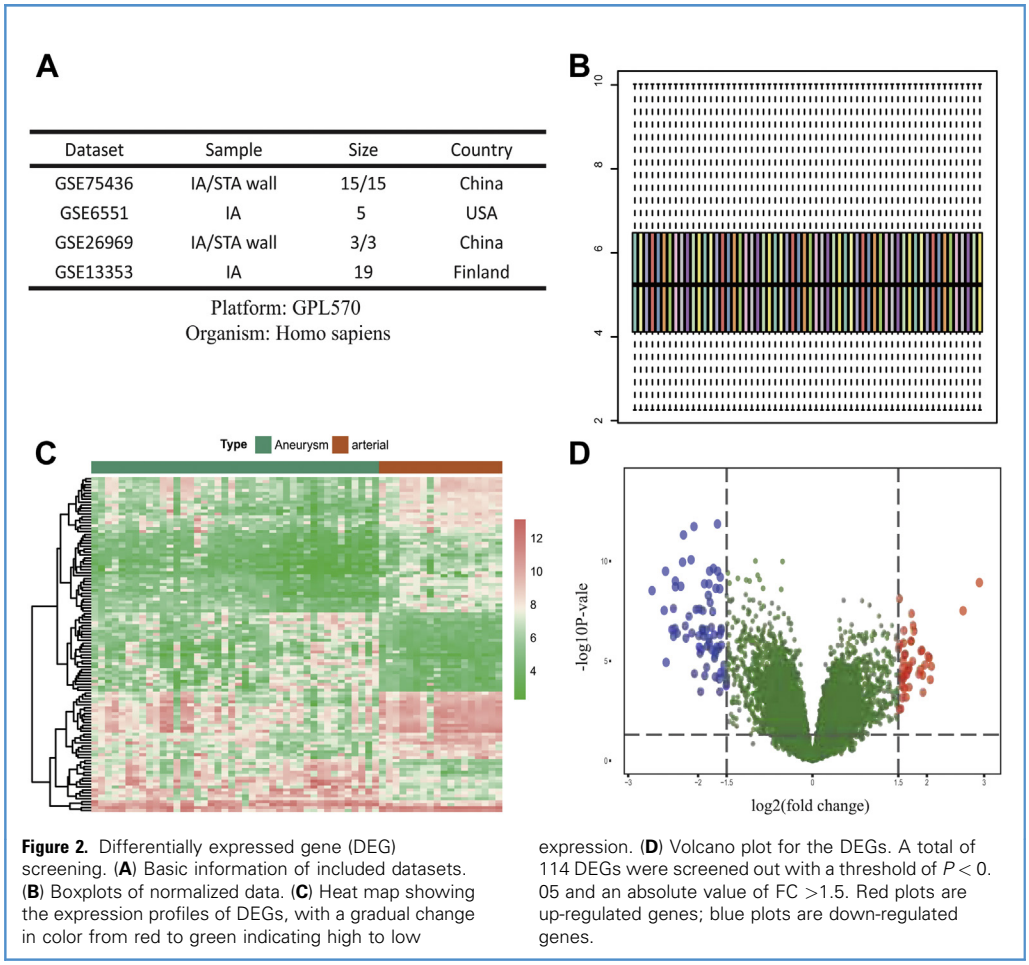

Figure 2. Differentially expressed gene (DEG) screening. (**A**) Basic information of included datasets. (**B**) Boxplots of normalized data. (**C**) Heat map showing the expression profiles of DEGs, with a gradual change in color from red to green indicating high to low expression. (**D**) Volcano plot for the DEGs. A total of 114 DEGs were screened out with a threshold of $P < 0.05$ and an absolute value of FC >1.5. Red plots are up-regulated genes; blue plots are down-regulated genes.

Computing, Vienna, Austria). Once DEGs were identified, functional and pathway enrichment analyses, gene set enrichment analysis (GSEA), and protein–protein interaction (PPI) network analysis were used to evaluate connections among DEGs and determine DEG interactions on the molecular level (Figure 1).

Microarray Data Preprocessing and DEG Screening

Because GSE75436, GSE6551, GSE26969, and GSE13353 are from the same platform, raw data were merged and normalized, and a batch effect was removed using the SVA package.[6] Probes without a corresponding gene symbol were then filtered out, and the average value of the gene symbols were calculated with multiple probes. Between the 2 groups, the Linear Models for Microarray Data Analysis (limma) package[7] was used to screen the DEGs. Threshold values were set as $P < 0.05$ and absolute value of logFC >1.5.

Functional Analysis of DEGs

Today, the most widely trusted gene function knowledge bases are Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). In this study, we used the clusterProfiler package[8] to analyze function profiles of genes and gene clusters to identify major biological functions of genes. All DEGs went through KEGG pathway analyses and GO analyses, including biological process, cellular component, and molecular function using clusterProfiler.

GSEA

GSEA helps determine whether distinct sets of genes have significant differences using computational methods. We performed GSEA analysis using the R package clusterProfiler. Differences were considered statistically significant at |NES| >1, nominal $P < 0.05$, and false discovery rate q value <0.25. Then the genes were evaluated with the clusterProfiler package to analyze GO biological process and the KEGG pathway. The cutoff value for significant enrichment was set at $P < 0.05$.

PPI Analysis

To examine the function of genes at the protein level and to explore the core genes involved in cellular processes of IA formation, STRING (Search Tool for the Retrieval of Interacting Genes/Proteins; http://string-db.org/) was used to look for experiment-validated PPIs among the common DEGs and develop networks for annotation of structural and functional properties of the proteins. In PPI analysis, the threshold of confidence score >0.4 was set to be default. We used a k-core analysis to assess

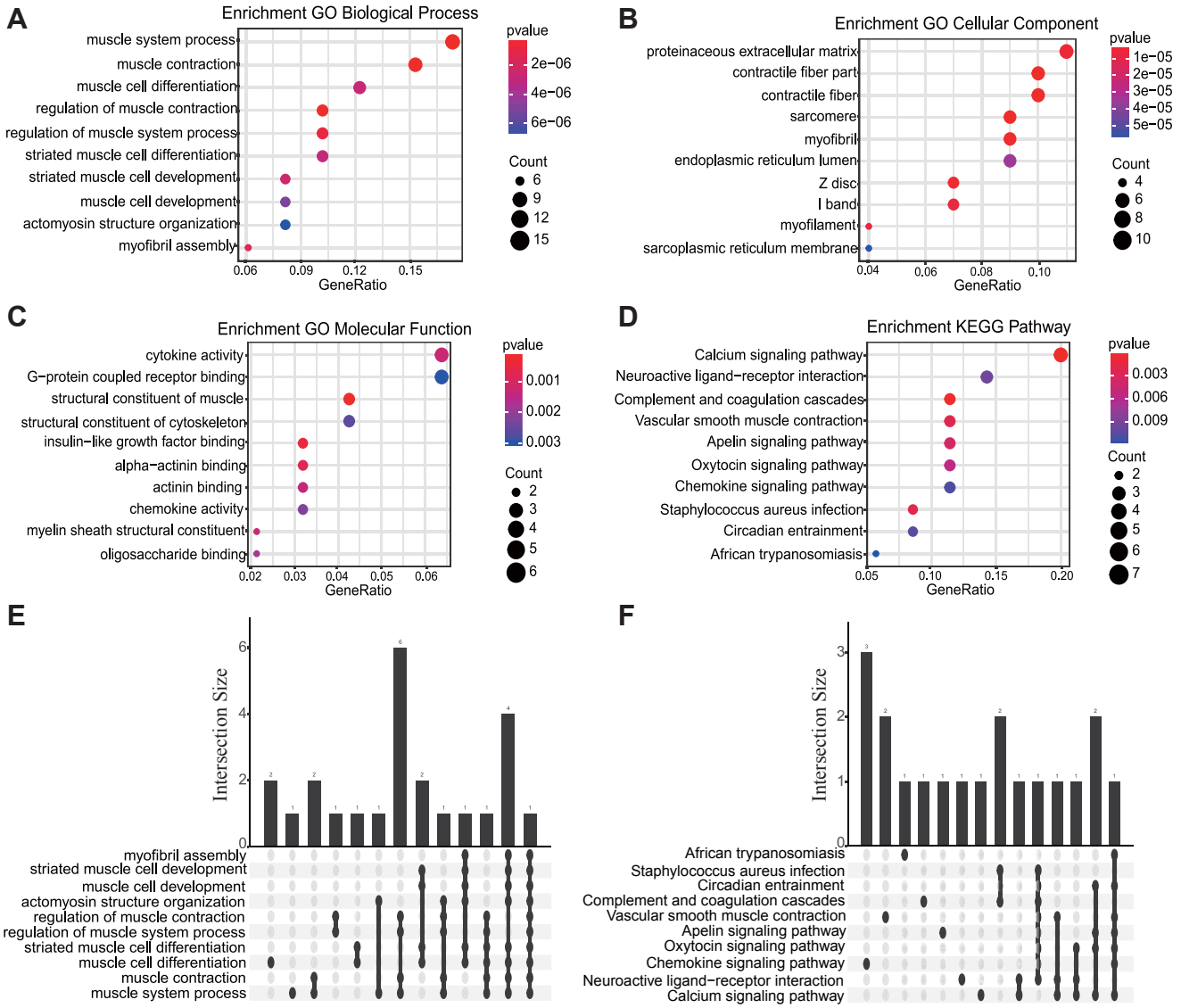

Figure 3. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of common differentially expressed genes (DEGs) in the IA group. (**A**) GO enrichment in biological function with the top 10 terms. (**B**) GO enrichment in the cellular component with the top 10 terms. (**C**) GO enrichment in molecular function with the top 10 terms. (**D**) KEGG pathway enrichment analysis of common DEGs with the top 10 terms. (**E**) Venn diagram showing overlap in GO biological process. (**F**) Venn diagram displaying overlap in the KEGG pathway analysis.

important subnetworks that were created by recursively removing vertices with degrees less than k from the network. The results were visualized using Cytoscape software, and the MCODE package was adopted to search for clusters within the PPI network.

RESULTS

Data Preprocessing and DEG Screening

In total, we recruited 42 cases of IAs and 18 cases of normal arteries as controls (Figure 2A). Similar distributions were seen in normalized data after preprocessing, supporting the reliability of our data (Figure 2B). On filtering with the limma package, 43 up-regulated genes and 71 down-regulated genes were recognized (Figure 2C). To obtain a full picture of different gene expressions in all cases, we drew a volcano plot (Figure 2D). The threshold was set to be $P < 0.05$ and absolute value of FC >1.5, with up-regulated genes in red and down-regulated genes in green.

Functional Analysis of DEGs

We examined the functions of DEGs to gain insight into disease progression in IAs. GO analysis and KEGG pathway analysis were

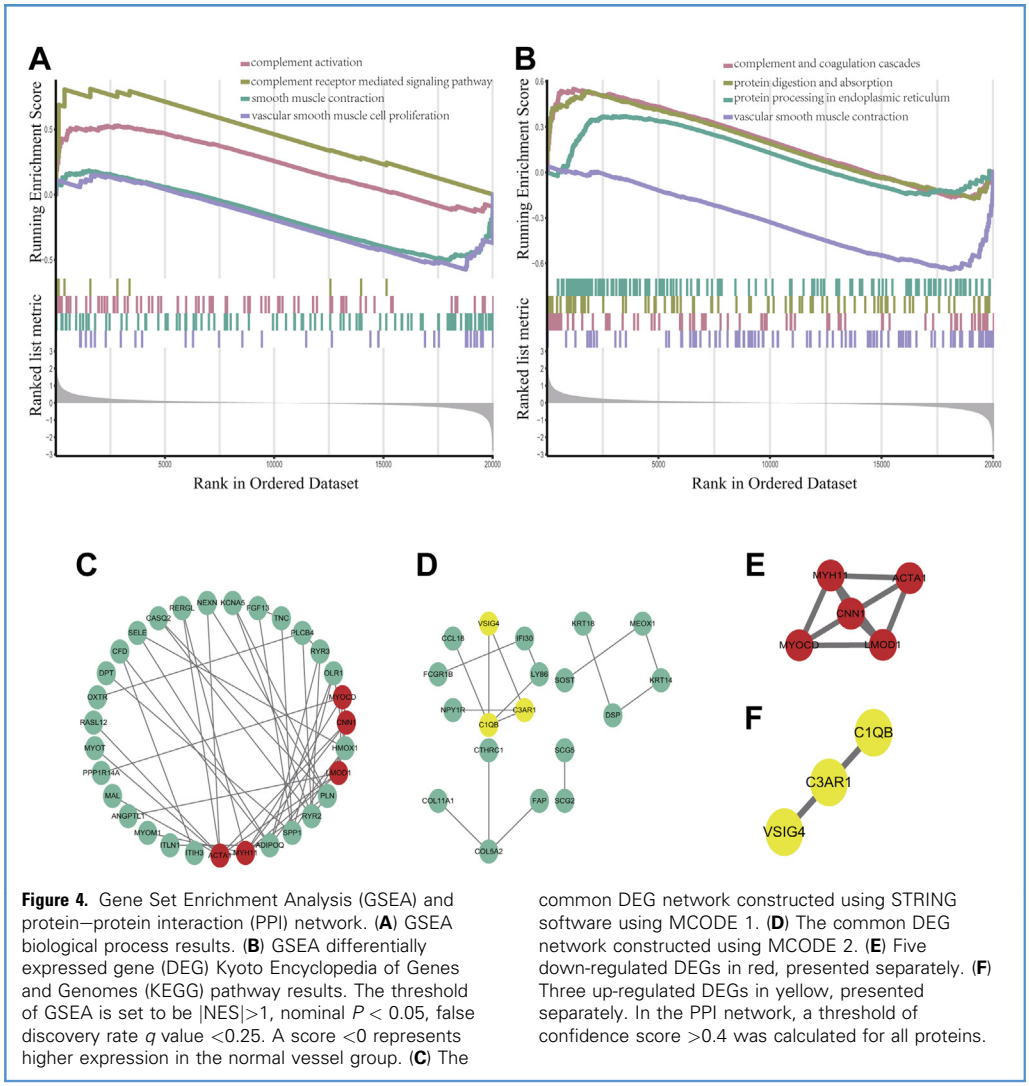

Figure 4. Gene Set Enrichment Analysis (GSEA) and protein–protein interaction (PPI) network. (**A**) GSEA biological process results. (**B**) GSEA differentially expressed gene (DEG) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway results. The threshold of GSEA is set to be |NES|>1, nominal $P < 0.05$, false discovery rate q value <0.25. A score <0 represents higher expression in the normal vessel group. (**C**) The common DEG network constructed using STRING software using MCODE 1. (**D**) The common DEG network constructed using MCODE 2. (**E**) Five down-regulated DEGs in red, presented separately. (**F**) Three up-regulated DEGs in yellow, presented separately. In the PPI network, a threshold of confidence score >0.4 was calculated for all proteins.

used to sort out DEGs. Among GO biological processes, the most overrepresented are muscle system process, muscle contraction, muscle cell differentiation, and the like. In the GO cellular component, the most enriched are proteinaceous extracellular matrix, contractile fiber, and so on. In GO molecular function, structural constituent of muscles and the cytoskeleton are notable (Figure 3A–C). In the KEGG pathway analysis, DEGs were notably enriched in complement and coagulation cascades, vascular smooth muscle contraction, the calcium signaling pathway, and others (Figure 3D). Venn diagrams were used to present the overlapping relationships in biological processes and KEGG pathway results (Figure 3E and F).

GSEA

In GSEA biological processes results (Figure 4A), complement activation and complement receptor mediated signaling pathway showed higher expression in the IA group, whereas smooth muscle contraction and vascular smooth muscle cell proliferation were more frequent in the normal vessel control group. In GSEA KEGG results (Figure 4B), vascular smooth muscle contraction was more enriched in the normal blood vessel group, whereas complement and coagulation cascades, protein digestion and absorption, and protein processing in the endoplasmic reticulum were increased in the IA group. Detailed GSEA results are presented in Table 1.

PPI Network of the Common DEGs

The online STRING database was recruited to construct the PPI network. The medium confidence score (0.4) was set to evaluate PPIs of the DEGs. The PPI network included 50 nodes and 60 edges. We used MCODE to analyze the network and came to 2 cluster results named MCODE 1 and MCODE 2. In MCODE analysis 1, 5 DEGs (*MYH11*, *CNN1*, *MYOCD*, *ACTA1*, and *LMOD1*) were down-regulated in IA patients (Figure 4C–E, marked in red); in MCODE 2, 3 DEGs (*C1QB*, *C3AR1*, and *VSIG4*) were up-regulated in these patients (Figure 4D–F, in yellow). Identified key genes and respective biological functions are presented in Table 2.

Table 1. Details of Gene Set Enrichment Analysis Results

ID	Description	Set Size	Enrichment	*P* Value
GO:1990874	Vascular smooth muscle cell proliferation	36	−0.57	0.012
GO:0006939	Smooth muscle contraction	99	−0.50	0.004
GO:0006956	Complement activation	78	1.73	0.002
GO:0002430	Complement receptor−mediated signaling pathway	10	1.72	0.012
hsa04270	Vascular smooth muscle contraction	112	−2.17	0.002
hsa04610	Complement and coagulation cascades	77	1.82	0.002
hsa04974	Protein digestion and absorption	85	1.79	0.002
hsa04141	Protein processing in endoplasmic reticulum	157	1.35	0.021

DISCUSSION

In contrast to terminally differentiated muscle cells, such as skeletal and cardiac muscle cells, vascular SMCs exhibit significant plasticity in response to injury and inflammation.[9,10] The phenotype of SMCs is not persistent[11]; under certain conditions, SMCs can have phenotype alteration from contraction-related functions to a proinflammatory and matrix remodeling phenotype.[12,13] This critical process is a key foundation of IA pathology.[5] Once altered, the border between SMCs and inflammatory cells, such as macrophages, is blurred. SMC phenotypic modulation to an inflammatory state may promote IA.[14] In extreme situations, such as endothelial cell injury and arterial wall inflammation, SMCs dedifferentiate.[9] Specifically, SMCs undergo phenotypic modulation from a differentiated phenotype related mainly to contraction to an undifferentiated proinflammatory pro−matrix remodeling phenotype.[12,15] With immunohistochemistry, phenotypic modulation of SMCs was confirmed in IA walls.[16] Driven by tumor necrosis factor α, the expression of contractile

Table 2. Information of 8 Key Genes

Gene Symbol	Gene Title	*P* Value	Log FC	Related Biological Process
MYH11	Myosin heavy chain 11	4.71e-06	−1.70	GO:0006936 muscle contraction GO:0006939 smooth muscle contraction GO:0048251 elastic fiber assembly
CNN1	Calponin 1	1.95e-06	−1.98	GO:0006940 regulation of smooth muscle contraction GO:0031032 actomyosin structure organization GO:1904706 negative regulation of vascular smooth muscle cell proliferation
MYOCD	Myocardin	4.33e-05	−2.06	GO:0045987 positive regulation of smooth muscle contraction GO:0051152 positive regulation of smooth muscle cell differentiation GO:1900239 regulation of phenotypic switching
ACTA1	Actin alpha 1	7.09e-07	−2.21	GO:0006936 muscle contraction GO:0009991 response to extracellular stimulus GO:0030049 muscle filament sliding
LMOD1	Leiomodin 1	2.91e-07	−1.91	GO:0006936 muscle contraction GO:0030239 myofibril assembly GO:0030838 positive regulation of actin filament polymerization
C1QB	B-chain of complement component 1q	6.67e-04	1.64	GO:0006508 proteolysis GO:0006958 complement activation, classical pathway GO:0045087 innate immune response
C3AR1	Complement component 3a receptor 1	5.03e-06	1.61	GO:0002430 complement receptor mediated signaling pathway GO:0006954 inflammatory response GO:0030449 regulation of complement activation
VSIG4	V-set and immunoglobulin-domain containing 4	2.42e-04	1.60	Complement activation, alternative pathway (GO:0006957) Negative regulation of interleukin-2 production (GO:0032703) Negative regulation of macrophage activation (GO:0043031)

genes/proteins, such as smooth muscle myosin heavy chain (SM-MYH), was significantly decreased in IA samples compared with control cerebral arteries.[16-19] These data show that SMCs play an important role in initiating or at least promoting the inflammatory and degenerative response that drives IA formation.

In this study, to recognize possible genes related to IA pathogenesis, we compared microarray data in IAs and normal blood vessels. In the IA samples, 114 DEGs were screened out. The enrichment analysis demonstrated that DEGs were primarily enriched in biological processes, including muscle cell development, differentiation, and contractile function. In GO cellular component enrichment analysis, the most enriched component was proteinaceous extracellular matrix, whereas in GO molecular function analysis, it was structural constituent of muscle. In KEGG pathway analysis, DEGs were enriched in vascular smooth muscle contraction.

To provide evidence from another perspective, we performed GSEA in our dataset. Complement coagulation cascades, protein digestion and absorption, and protein processing in endoplasmic reticulum were enriched in the IA group. These processes may relate to SMCs' phenotypic modulation into a promatrix type. Meanwhile, vascular smooth muscle contraction, having a negative running enrichment score, was more enriched in the normal blood vessel group. The result indicates the importance of smooth muscle contraction in preventing aneurysm pathogenesis. Also, complement activation and the complement receptor-mediated signaling pathway were enriched in the IA group with a positive enrichment score, whereas smooth muscle contraction and vascular smooth muscle cell proliferation were enriched in the control group. These results also suggest that increased inflammation and decreased smooth muscle function are involved in IA pathogenesis. Our findings are in accordance with previously reported data on the role of vascular SMCs in IAs pathogenesis.

Here we further discuss the 8 key genes (*MYH11*, *CNN1*, *MYOCD*, *ACTA1*, *LMOD1*, *C1QB*, *C3AR1*, and *VSIG4*), starting with the decreased ones. *MYH11*, belonging to the myosin heavy chain family, was found to be down-regulated in IAs process in our study. The myosin heavy chain family, including the aforementioned SM-MYH, is reportedly crucial in many diseases, including IAs.[16,17,20] In a DNA methylation study using DNA microarray data analysis, *MYH11* was reportedly associated with the occurrence and development of IAs.[21] In another case report, a 6-month-old girl with a *MYH11* mutation was reported to have rapid de novo aneurysm formation after clipping of a ruptured middle cerebral artery aneurysm.[22] These studies support the idea that *MYH11* is important in the pathogenesis of IAs. Calponin 1 (*CNN1*) encoded by the *CNN1* gene is an actin filament-associated regulatory protein specifically expressed in differentiated mature SMCs, suggesting a function in contraction.[23] During postnatal development, *CNN1* is up-regulated in smooth muscle tissues.[24] There has been little research on *CNN1* and IAs to date. One study focusing on Polycystin-1 (PC1) showed that PC1 down-regulation in vascular smooth muscle cells promotes the expression of CNN1 protein, which may be a key pathophysiological factor in vascular diseases.[25] Myocardin (*MYOCD*) is a smooth muscle and cardiac muscle-specific transcriptional coactivator of serum response factor.[26] *MYOCD* has an important during postnatal development, and a lack of *MYOCD* in SMCs induces endoplasmic reticulum stress, autophagy, and apoptosis.[27] One study showed that *MYOCD* can be targeted by miR-9, and the dysregulation of miR-9 can lead to IAs.[28] In another study, *MYOCD* was identified as a key regulator of vascular SMC contractility and a crucial factor in IA progression.[29] Known to cause muscle formation, *ACTA1* is expressed mostly in skeletal muscle.[30] Meanwhile, a vascular SMC-specific isoform of actin alpha (*ACTA2*) causes a variety of vascular diseases, including thoracic aortic aneurysm and dissection, premature coronary artery disease, stroke, and moyamoya disease[31]; however, the role of *ACTA2* in IAs remains controversial. *LMOD1* is preferentially expressed in vascular and visceral SMCs.[32] Loss of *LMOD1* results in a reduction of filamentous actin, elongated cytoskeletal dense bodies, and impaired intestinal smooth muscle contractility.[33] *LMOD1* and *MYH11*, both highly specific contractile genes for smooth muscle lineages, have been associated with megacystis microcolon intestinal hypoperistalsis syndrome.[33,34] Considering the important role of *MYH11* in the pathogenesis of IA, it is reasonable to hypothesize that the loss of *LMOD1* has a role in IA pathogenesis as well. However, no direct study focusing on the association between *LMOD1* and IA has been reported to date.

Our analysis identified 3 up-regulated genes: *C1QB*, *C3AR1*, and *VSIG4*. *C1QB* encodes the B-chain of complement component 1q (C1q), a protein complex involved in the complement system.[35] *C3AR1* encodes the complement component 3a receptor 1 (*C3AR1*), a G protein—coupled receptor protein involved in the complement system.[36] The v-set and immunoglobulin-domain containing 4, a protein encoded by *VSIG4*, relates structurally to the B7 family of immune regulatory proteins and is a receptor for the complement component 3 fragments C3b and iC3b.[37] *VSIG4* binds to the C3b/iC3b and activates macrophage immunity.[38] Increased expression levels of *C1QB*, *C3AR1*, and *VSIG4* are related to an enhanced complement system.

In summary, SMC phenotypic modulation from a contractile state to a proinflammatory state is a key step in forming IAs. As supported by our GSEA study, this process can be characterized by decreased expression of SMC contractile genes and increased expression of proinflammatory, pro—matrix remodeling genes. By analyzing data from multiple datasets, we found 5 down-regulated genes (*MYH11*, *CNN1*, *MYOCD*, *ACTA1*, and *LMOD1*) related to contractibility or stability of SMCs, along with 3 up-regulated genes (*C1QB*, *C3AR1*, and *VSIG4*) involved in complement system regulation. The key genes that we have identified here are in accordance with the prevailing SMC phenotypic modulation theory. Notably, given the reported association between a *MYH11* mutation and de novo aneurysm formation, this relationship between *MYH11* and IA merits further investigation. Moreover, because *LMOD1* and *MYH11* have been reported to play roles in megacystis microcolon intestinal hypoperistalsis syndrome, *LMOD1* may have a more significant role in the pathogenesis of IA compared with other DEGs.

This study has identified potential genes that may play crucial roles in IA pathogenesis, but is limited by the lack of basic research

verification, owing to limited resources. In addition, the results are biased to some extent, because the database that we used did not provide any clinical features, such as age, sex, different types of aneurysms, and so on. It is possible that IA samples can be further stratified into separate groups based on clinical characteristics, especially by type of aneurysms (e.g., blood blister-like aneurysm). Therefore, further research focusing on the aforementioned key genes and different types of aneurysms will provide more insight into this area to improve the treatment of IA.

ACKNOWLEDGMENTS

We thank Bing Jiang and Zhixiong Liu for their assistance with this research.

REFERENCES

1. Keedy A. An overview of intracranial aneurysms. *Mcgill J Med*. 2006;9:141-146.
2. Xia ZW, Liu XM, Wang JY, et al. Coiling is not superior to clipping in patients with high-grade aneurysmal subarachnoid hemorrhage: systematic review and meta-analysis. *World Neurosurg*. 2017;98:411-420.
3. Jeon TY, Jeon P, Kim KH. Prevalence of unruptured intracranial aneurysm on MR angiography. *Korean J Radiol*. 2011;12:547-553.
4. Yang P, Schmit BM, Fu C, et al. Smooth muscle cell-specific Tgfbr1 deficiency promotes aortic aneurysm formation by stimulating multiple signaling events. *Sci Rep*. 2016;6:35444.
5. Starke RM, Chalouhi N, Ding D, et al. Vascular smooth muscle cells in cerebral aneurysm pathogenesis. *Transl Stroke Res*. 2014;5:338-346.
6. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics*. 2012;28:882-883.
7. Ritchie ME, Phipson B, Wu D, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43:e47.
8. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*. 2012;16:284-287.
9. Alexander MR, Owens GK. Epigenetic control of smooth muscle cell differentiation and phenotypic switching in vascular development and disease. *Annu Rev Physiol*. 2012;74:13-40.
10. Yuan L, Shu C, Zhou X, et al. Radiation suppresses neointimal hyperplasia through affecting proliferation and apoptosis of vascular smooth muscle cells. *J Vasc Access*. 2018;19:153-161.
11. Huang F, Yin J, Li K, et al. GPR30 decreases with vascular aging and promotes vascular smooth muscle cells maintaining differentiated phenotype and suppressing migration via activation of ERK1/2. *Onco Targets Ther*. 2016;9:3415-3422.
12. Ali MS, Starke RM, Jabbour PM, et al. TNF-α induces phenotypic modulation in cerebral vascular smooth muscle cells: implications for cerebral aneurysm pathology. *J Cereb Blood Flow Metab*. 2013;33:1564-1573.
13. Tai S, Hu XQ, Peng DQ, Zhou SH, Zheng XL. The roles of autophagy in vascular smooth muscle cells. *Int J Cardiol*. 2016;211:1-6.
14. Chalouhi N, Ali MS, Jabbour PM, et al. Biology of intracranial aneurysms: role of inflammation. *J Cereb Blood Flow Metab*. 2012;32:1659-1676.
15. Starke RM, Ali MS, Jabbour PM, et al. Cigarette smoke modulates vascular smooth muscle phenotype: implications for carotid and cerebrovascular disease. *PLoS One*. 2013;8:e71954.
16. Nakajima N, Nagahiro S, Sano T, Satomi J, Satoh K. Phenotypic modulation of smooth muscle cells in human cerebral aneurysmal walls. *Acta Neuropathol*. 2000;100:475-480.
17. Kilic T, Sohrabifar M, Kurtkaya O, et al. Expression of structural proteins and angiogenic factors in normal arterial and unruptured and ruptured aneurysm walls. *Neurosurgery*. 2005;57:997-1007 [discussion: 997-1007].
18. Pera J, Korostynski M, Krzyszkowski T, et al. Gene expression profiles in human ruptured and unruptured intracranial aneurysms: what is the role of inflammation? *Stroke*. 2010;41:224-231.
19. Sibon I, Mercier N, Darret D, Lacolley P, Lamazière JM. Association between semicarbazide-sensitive amine oxidase, a regulator of the glucose transporter, and elastic lamellae thinning during experimental cerebral aneurysm development: laboratory investigation. *J Neurosurg*. 2008;108:558-566.
20. Zhang S, Zhou X, Liu S, et al. MYH9-related disease: description of a large Chinese pedigree and a survey of reported mutations. *Acta Haematol*. 2014;132:193-198.
21. Chen L, Fan Y, Wan J. Screening of key genes of unruptured intracranial aneurysms by using DNA microarray data analysis techniques. *Genet Mol Res*. 2014;13:758-767.
22. Ravindra VM, Karsy M, Schmidt RH, Taussky P, Park MS, Bollo RJ. Rapid de novo aneurysm formation after clipping of a ruptured middle cerebral artery aneurysm in an infant with an MYH11 mutation. *J Neurosurg Pediatr*. 2016;18:463-470.
23. Takahashi K, Abe M, Hiwada K, Kokubu T. A novel troponin T-like protein (calponin) in vascular smooth muscle: interaction with tropomyosin paracrystals. *J Hypertens Suppl*. 1988;6:S40-43.
24. Hossain MM, Hwang DY, Huang QQ, Sasaki Y, Jin JP. Developmentally regulated expression of calponin isoforms and the effect of h2-calponin on cell proliferation. *Am J Physiol Cell Physiol*. 2003;284:C156-C167.
25. Feng J, Ge S, Zhang L, Che H, Liang C. Aortic dissection is associated with reduced polycystin-1 expression, an abnormality that leads to increased ERK phosphorylation in vascular smooth muscle cells. *Eur J Histochem*. 2016;60:2711.
26. Wang DZ, Li S, Hockemeyer D, et al. Potentiation of serum response factor activity by a family of myocardin-related transcription factors. *Proc Natl Acad Sci U S A*. 2002;99:14855-14860.
27. Huang J, Wang T, Wright AC, et al. Myocardin is required for maintenance of vascular and visceral smooth muscle homeostasis during postnatal development. *Proc Natl Acad Sci U S A*. 2015;112:4447-4452.
28. Luo J, Jin H, Jiang Y, Ge H, Wang J, Li Y. Aberrant expression of microRNA-9 contributes to development of intracranial aneurysm by suppressing proliferation and reducing contractility of smooth muscle cells. *Med Sci Monit*. 2016;22:4247-4253.
29. Zhang M, Ren Y, Wang Y, et al. Regulation of smooth muscle contractility by competing endogenous mRNAs in intracranial aneurysms. *J Neuropathol Exp Neurol*. 2015;74:411-424.
30. Bandman E. Contractile protein isoforms in muscle development. *Dev Biol*. 1992;154:273-283.
31. Yuan SM. α-smooth muscle actin and ACTA2 gene expressions in vasculopathies. *Braz J Cardiovasc Surg*. 2015;30:644-649.
32. Conley CA, Fritz-Six KL, Almenar-Queralt A, Fowler VM. Leiomodins: larger members of the tropomodulin (Tmod) gene family. *Genomics*. 2001;73:127-139.
33. Halim D, Wilson MP, Oliver D, et al. Loss of LMOD1 impairs smooth muscle cytocontractility and causes megacystis microcolon intestinal hypoperistalsis syndrome in humans and mice. *Proc Natl Acad Sci U S A*. 2017;114:E2739-2747.
34. Gauthier J, Ouled Amar Bencheikh B, Hamdan FF, et al. A homozygous loss-of-function variant in MYH11 in a case with megacystis-microcolon-intestinal hypoperistalsis syndrome. *Eur J Hum Genet*. 2015;23:1266-1268.
35. Sellar GC, Blake DJ, Reid KB. Characterization and organization of the genes encoding the A-, B- and C-chains of human complement subcomponent C1q. The complete derived amino acid sequence of human C1q. *Biochem J*. 1991;274(Pt 2):481-490.

36. Roglic A, Prossnitz ER, Cavanagh SL, Pan Z, Zou A, Ye RD. cDNA cloning of a novel G protein-coupled receptor with a large extracellular loop structure. *Biochim Biophys Acta*. 1996;1305:39-43.

37. Vogt L, Schmitz N, Kurrer MO, et al. VSIG4, a B7 family-related protein, is a negative regulator of T cell activation. *J Clin Invest*. 2006;116:2817-2826.

38. Helmy KY, Katschke KJ Jr, Gorgani NN, et al. CRIg: a macrophage complement receptor required for phagocytosis of circulating pathogens. *Cell*. 2006;124:915-927.

Conflict of interest statement: This work was supported by the National Natural Science Foundation of China (NO.81703622; NO.81873756), China Postdoctoral Science Foundation (NO. 2018M633002), and Hunan Provincial Natural Science Foundation of China (NO.2018JJ3838).

Received 19 April 2019; accepted 14 June 2019

Citation: World Neurosurg. (2019) 131:e23-e31.
https://doi.org/10.1016/j.wneu.2019.06.110

Journal homepage: ***www.journals.elsevier.com/world-neurosurgery***

Available online: ***www.sciencedirect.com***